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Recombinant bFGF Promotes Wound Healing after Ballistic Injury

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The effects of basic fibroblast growth factor (bFGF) on promoting wound healing were studied in an attempt to discover new methods of treating ballistic injury. The effects of recombinant bFGF and factors influencing these effects in rabbits with ballistic injury were studied. The right hind legs of rabbits were shot with a pistol, after which bFGF was used to treat the wound tracks. The time required for ballistic wound track healing was shorter in the treatment group than in the control group (37.9 ± 5.6 days vs 53.3 ± 8.3 days). Histological examination confirmed that bFGF stimulates the formation of granulation tissue, the regeneration of capillaries and the proliferation of fibroblasts. Immunohistochemical staining of the extracellular matrix of the granulation tissue showed that the amounts of fibronectin, laminin and type III collagen were higher in the treatment group. Heparin and gentamycin each enhanced the action of bFGF in wound healing. In order to maintain bFGF activity, it is essential that the wound track is kept free of infection and all necrotic tissue be excised. ***(Asian J. Surgery 1997;20(4):320–323)***

Fibroblast growth factors (FGFs) have been used extensively in the treatment of surgical wounds,[1] but not for ballistic injuries. In ballastic injuries, the dynamics of tissue damage is more complex and wound healing by second intention is slower than in other types of injuries. At present, attempts to enhance wound healing are limited. In this study, the effects of recombinant basic FGF (bFGF), as well as factors which influence its wound healing activity, were observed, in an attempt to find newer methods to treat wound tracks caused by ballistic injuries.

Materials and Methods

One hundred and thirty New Zealand rabbits (weight 2.5–3.0 kg) were divided randomly into treatment (n = 98) and control (n = 32) groups. The treatment group was further divided into three groups: bFGF (1.5 μg) alone (n = 34) at a concentration of 1.0 mg/ml dissolved in normal saline, bFGF (1.5 μg) + heparin (0.2 μg /0.5 ml normal saline (n = 32) and bFGF (1.5 μg) + gentamicin (200,000 U) (n = 32). The rabbits were anaesthetised with 8 mg/kg pentobarbital sodium. Animals were subjected to a ballistic injury to the right hind leg with a "54" model pistol using a 5.56 mm M193 type of cartridge. Immediately after injury, the bleeding was stopped by compression and the legs were bound. The wounds were cleaned by excising tissue debris and removing blood clots, and washed with 1% benzyl bromide, 3% hydrogen peroxide and normal saline. The wounds were then packed with gauze soaked with normal saline. From the fifth day after injury, gauze impregnated with bFGF, bFGF + heparin or bFGF + gentamicin were applied to the wounds in the treatment groups daily. Gauze soaked with normal saline was used in the control group. Simple support treatment was given for injury.

Changes in the volume of the wound track, which reflect the amount of granulation tissue being deposited and the time required for the wound to heal, were

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determined by measuring the amount of normal saline the wound could hold. The number and types of cells in the wound discharge were counted and identified using Wright's stain. Aerobic and anaerobic bacteria were cultured from the discharge. On the first, second and third week after injury, animals were sacrificed in groups of four. After the animals were killed, the growth of granulation tissue was recorded and tissue fibronectin, laminin, type III collagen, DNA and argylophilic nucleolar organism region associated protein[2] (AgNORs) were observed at different times after injury with immunohistochemical stains (All antibodies were purchased from DAKO Glostrup, Demark). Every day before the wounds were treated, pH paper strips were placed on the wound tract to determine the pH of the wound. All data are expressed as the mean ± standard error. The comparisons between the two groups were made using the *t*-test.

Results

Wound track healing

On the first day after injury, the volume of the wound track was 15.3 ± 2.5 cm^3 in the treatment group and 14.9 ± 2 cm^3 in the control group. The number of cells in the discharge was 2.0 ± 0.5 / field (x400). In the treatment group, one week after treatment with bFGF alone, the volume of the wound track was reduced to a quarter of the initial value, there was little discharge, and the cells present were mainly neutrophils and monocytes. The floor of the wound was red and the new granulation tissue bled easily. After three weeks, the number of cells in the discharge was 5.0 ± 2.5/field (x400). The signs of complete wound healing would be that the wound tract is closed and epidermal cells have completely covered the entrance and exit of the wound tract. During the fifth week after injury, the wound tracks appeared to heal and epidermalise. The time required for wound healing was 37.9 ± 5.6 days.

In the control group, it took two weeks for the volume of the wound track to be reduced to a quarter. The discharge was more than in the treatment group and cells were mainly neutrophils. The newly laid granulation tissue was dark red. After three weeks, the number of cells in the discharge was 40.5 ± 12.5/field (x400). On the 50th day after injury, the wound track appeared to heal and epidermalise. The time required for the wounds to heal was 53.3 ± 8.3 days, which was significantly longer than in the treatment group ($p < 0.01$). The kinetic changes in ballistic wound track healing are shown in Figure 1.

Growth of granulation tissue

In the treatment group, after one week of using bFGF, fibroblasts and capillaries began to proliferate and regenerate. By the second week, the number of fibroblasts and capillaries increased significantly. Fibroblasts stained strongly with anti-DNA antibody and antibodies against desmin intermediate filament proteins.[3] Some of the components of the extracellular matrix (laminin, fibronectin, type III collagen) increased significantly. The number of AgNORs granulations in the nucleus was 3.21 ± 0.33.

In the control group, fibroblasts and capillaries began to proliferate and regenerate during the second week after injury. Fibroblasts were weakly positive to anti-DNA antibody and were negative for desmin intermediate filament proteins (Table 1). The contents of the extracellular matrix was less. The number of AgNORs granulations in nucleus was 2.21 ± 0.2.

Factors influencing the action of bFGF in wound healing

The time required for wound track healing was approximately 6.5 days less in the bFGF + heparin group than in the group using bFGF only ($p < 0.01$) (Table 2).

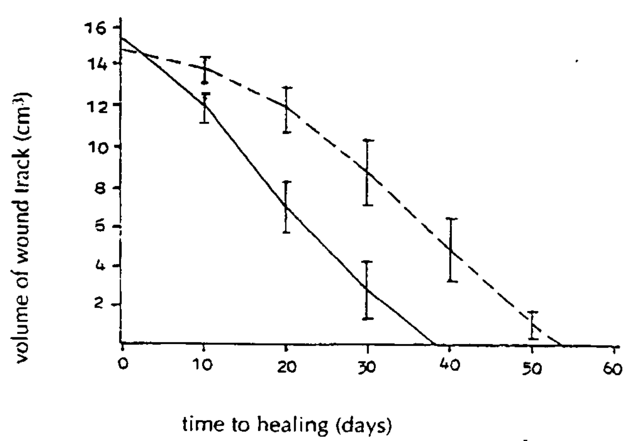

Figure 1. Kinetic changes in wound track healing. --- = control; — = treatment.

Table 1. Histological features of rabbit granulation tissue during the third week of wound healing after a ballistic injury

Group	No. of rabbits	Capillaries	Fibroblasts	Collagen Type III	DNA content in fibroblasts
Treatment	12				
bFGF	4	++	++	+ ~ ++	++ ~ +++
bFGF + heparin	4	+++	++	++	+++
bFGF + gentamicin	4	++	++ ~ +++	+ ~ ++	++ ~ +++
Control	4	+ ~ ++	+ ~ ++	+	+ ~ ++

10 fields were observed per slide: capillaries (having a distinct lumen): 0.5(+), 5–10(++), >10(+++); fibroblasts: 0–30(+), 31–50(++), >51(+++); collagen type III: according to stain intensity; DNA content: by number of granules in nucleus and cytoplasm: 0–30(+), 31 = 50(++), 51(+++). FGF = fibroblast growth factor.

Histological examination showed that heparin enhances the growth of granulation tissue, as well as the regeneration of capillaries and proliferation of fibroblasts.

The time for wound track healing in the group with bFGF + gentamicin was approximately 5.3 days less than that of the group with bFGF only ($p < 0.01$). During the first week after using bFGF + gentamicin, the wound track was infected, mainly with *Staphylococcus epidermidis* and *Escherichia coli*. The pH in the wound track was 7.0–7.4. When bFGF alone was used, the wound track became infected on the second day, mainly with *Pseudomonas aeruginosa*, and the pH in the wound track was 5.6–6.4.

Discussion

The results in this study confirm that bFGF promotes healing of ballistic wound tracks. It promotes growth of granulation tissue, stimulates proliferation of fibroblasts and the formation of capillaries, enhances synthesis and deposition of the extracellullar matrix (fibronectin, type III collagen and laminin) and stimulates fibroblasts and muscular cells to contract the wound tract. The old method of wound cleaning, surgery and the use of antibiotics may now be modified with effective measures for promoting wound healing with bFGF.

Factors that influence bFGF activity alter its structure.[4] Its effects on wound healing are influenced by the local wound environment,[5] for example, pH, infections, bleeding and necrosis around the wound track. bFGF is inactivated in acidic and hot environments. Another form of FGF, acid FGF (aFGF) is stable under acidic conditions but has lower activity than bFGF.[6] Therefore, we chose bFGF. Our results suggest that heparin can enhance the activity of bFGF in promoting wound track healing. Heparin has a high affinity for bFGF and protects it from being inactivated by enzymes, acid or heat.[7] If the wound track becomes infected, bacterial toxins and the acid environ-

Table 2. Effects of heparin or gentamicin on the action of basic fibroblast growth factor (bFGF) during wound healing

Group	No. of rabbits	Time to wound healing (days)	p
1.5 mg bFGF	30	37.9 ± 5.6	
1.5 mg bFGF + 0.2 μg heparin	30	31.4 ± 4.1	< 0.01
1.5 mg bFGF + 200,000 U gentamicin	30	32.6 ± 3.5	< 0.01

ment in the wound lead to loss of activity of bFGF. Our results support the proposal that infection should be controlled and necrotic tissue should be excised, in order to create an optimal condition for bFGF to promote wound healing.[7]

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